THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 274, No. 5, Issue of January 29, pp. 2766–2772, 1999
Printed in U.S.A.

Phytanic Acid Activates the Peroxisome Proliferator-activated Receptor α (PPARα) in Sterol Carrier Protein 2-/ Sterol Carrier Protein x-deficient Mice*

(Received for publication, September 18, 1998, and in revised form, October 30, 1998)

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We showed recently that a targeted null mutation in the murine sterol carrier protein 2-/sterol carrier protein x-gene (*Scp2*) leads to defective peroxisomal catabolism of 3,7,11,15-tetramethylhexadecanoic acid (phytanic acid), peroxisome proliferation, hypolipidemia, and enhanced hepatic expression of several genes that have been demonstrated to be transcriptionally regulated by the peroxisome proliferator-activated receptor α (PPARα). As a broad range of fatty acids activates PPARα *in vitro*, we examined whether the latter effects could be because of phytanic acid-induced activation of this transcription factor. Dietary phytol supplementation was used to modulate the concentration of phytanic acid in C57Bl/6 and *Scp2* (−/−) mice. We found that the serum concentrations of phytanic acid correlated well with the expression of genes encoding peroxisomal β-oxidation enzymes and liver fatty acid-binding protein, which have all been demonstrated to contain functionally active peroxisome proliferator response elements in their promoter regions. In accordance with these findings, a stimulating effect on acyl-CoA oxidase gene expression was also observed after incubation of the rat hepatoma cell line MH1C1 with phytanic acid. Moreover, reporter gene studies revealed that phytanic acid induces the expression of a peroxisome proliferator response element-driven chloramphenicol transferase reporter gene comparable with strong peroxisome proliferators. In addition, the ability of phytanic acid to act as an inductor of PPARα-dependent gene expression corresponded with high affinity binding of this dietary branched chain fatty acid to recombinant PPARα. We conclude that phytanic acid can be considered as a bona fide physiological ligand of murine PPARα.

Apart from serving as fuels in energy metabolism, fatty acids have been proposed to act as regulators in gene expression (reviewed in Ref. 1). Important roles in this process have been assigned to heterodimers consisting of peroxisome proliferator-activated receptor α (PPARα)[1] and retinoid X receptor α (RXRα), both of which are members of the superfamily of nuclear hormone receptors that function as ligand-dependent transcription factors (2–5). RXRα/PPARα heterodimers alter the transcription of target genes after binding to PPREs, which consist of a degenerate direct repeat of the recognition motif TGACCT spaced by 1 nucleotide (also called DR1 element) (2–4). Functionally active PPREs have been identified within the control regions of various genes implicated in lipid metabolism (overview in Ref. 6). The finding that several endogenous unsaturated fatty acids such as oleic acid, arachidonic acid, or linoleic acid activate PPARα *in vitro* supports the assumption that fatty acids could represent biological ligands for this nuclear hormone receptor (3, 7–10). It has been suggested that fatty acids regulate the transcription of genes involved in their own degradation by activating PPARα (7). On the other hand, a great number of chemically diverse peroxisome proliferators activate PPARα to a similar or even higher extent than all natural fatty acids, implying that the specificity of the fatty acid-mediated effect on PPARα may be low (9, 10). Therefore, it cannot be excluded that these agonists exert their effects indirectly by either being metabolized in the cell to an active form or by inducing the release or synthesis of a common endogenous ligand (9, 10).

Sterol carrier protein 2 (SCP2) and sterol carrier protein x (SCPx) are two peroxisomal proteins that are generated from the same gene via alternative transcription initiation (11). Based on *in vitro* data, it was assumed that SCP2 may play a role in intracellular cholesterol trafficking (reviewed in Ref. 12), whereas SCPx was identified as peroxisomal 3-ketoacyl-CoA thiolase with intrinsic lipid transfer activity (13). Recently, the phenotype of the SCP2/SCPx knockout mouse, Scp2 (−/−), did not provide evidence for a role of the gene in intracellular cholesterol trafficking but revealed instead defective peroxisomal degradation of certain natural methyl-branched fatty acyl-CoAs such as phytanic and pristanic acid, which are metabolized in peroxisomes. The metabolic abnormalities were associated with marked peroxisome proliferation, hypolipidemia, and enhanced expression of genes encoding peroxisomal β oxidation enzymes (14). Similar observations were made after feeding mice with fibrates (6, 15). Because the analysis of the PPARα knockout mice indicated that the fibrates exert their effects through PPARα (16), we investigated whether

* This work was supported by Deutsche Forschungsgemeinschaft Grants S.E. 459/2-2 (to U. S.) and SFB 310/A4 (to F. S.) and the Interdisziplinäres Klinisches Forschungszentrum of the Medical Faculty, University of Münster (Project A4). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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[1] The abbreviations used are: PPARα, peroxisome proliferator-activated receptor α; mPPARα, murine PPARα; RXRα, retinoid X receptor α; PPRE, peroxisome proliferator response element; SCP2, sterol carrier protein 2; SCPx, sterol carrier protein x; ACO, acyl-CoA oxidase; CAT, chloramphenicol transferase; GST, glutathione *S*-transferase; LBD, ligand binding domain; 9-*cis* RA, 9-*cis* retinoic acid.

phytanic acid could act as a fibrate-like natural agonist of PPARα. Phytanic acid was also identified as a weak agonist of RXRα, the obligate heterodimerization partner of PPARα (17, 18). Therefore, we further examined if the altered hepatic gene expression in our transgenic model could be because of phytanic acid-induced activation of RXRα. A synergistic effect on gene expression in the presence of ligands for both nuclear receptors has previously been described to occur *in vitro* (19, 20).

Our results reveal a strong correlation between phytanic acid serum concentrations and expression of genes encoding peroxisomal β-oxidation enzymes (acyl-CoA oxidase (ACO), peroxisomal bifunctional enzyme, peroxisomal 3-ketoacyl-CoA thiolase), and liver fatty acid-binding protein. In addition, we demonstrate that phytanic acid does not only bind to recombinant PPARα but also induces the expression of a PPRE-driven CAT reporter gene comparable with strong peroxisome proliferators. The identification of phytanic acid as a bona fide physiological ligand of PPARα is of special interest, as an accumulation of this dietary fatty acid is not only observed in Scp2 (−/−) mice but also in several inherited human diseases, *e.g.* Refsum disease and Zellweger syndrome (21).

EXPERIMENTAL PROCEDURES

Preparation of cDNA Probes and Northern Blot Analyses—Total RNA was isolated from mouse tissues or MH1C1 cells according to Chomczynski and Sacchi (22) followed by selection of poly(A)$^+$ RNA on oligo(dT) cellulose. Northern blots were hybridized with digoxigenin-labeled probes prepared by random priming using a commercially available kit (Boehringer Mannheim). All probes were obtained from a mouse liver cDNA library (Stratagene, Heidelberg, Germany) by polymerase chain reaction amplification with appropriate primers. Quantification was carried out relative to expression of glyceraldehyde-3-phosphate dehydrogenase mRNA. The membranes were rinsed twice in 0.1% SDS, 2× SSC (0.15 M NaCl and 0.015 M sodium citrate) at room temperature and then twice in 0.1% SDS, 0.5× SSC at 68 °C for 15 min. Bands were visualized using the chemiluminescence substrate CDP-Star (Tropix-Serva, Heidelberg, Germany) and quantified using a Bio-Imager BAS-KR 1500 (Fuji, Düsseldorf, Germany). DNA sequencing was performed on an automated laser fluorescence DNA sequencer (Amersham Pharmacia Biotech) to verify the identity of the polymerase chain reaction amplification products.

Cell Culture and Transfection—The rat hepatoma cell line MH1C1 was obtained from the DSMZ (Braunschweig, Germany) and cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and antibiotics. After washing with phosphate-buffered saline, cells were incubated for 72 h with 250 μM Wy 14,643, bezafibrate, or phytanic acid dissolved in Me_2SO (0.5% v/v). Wy 14,643 was obtained from Biomol (Hamburg, Germany), and bezafibrate and phytanic acid were obtained from Sigma. HepG2 cells were cultured in 6-well dishes with Dulbecco's modified Eagle's medium supplemented with 10% basal medium supplement artificial serum (Biochrom, Berlin, Germany) and grown to 70% confluency. Co-transfection of HepG2 cells with 1.5 μg/well pcDNA3-mPPARα (5, 23) and 1.5 μg of *pCAT-iPPRE* was performed with Fugene transfection reagent (Boehringer Mannheim). pcDNA3-mPPARα was a friendly gift from Dr. P. Holden (Zeneca), and the reporter gene construct *pCAT-iPPRE* was prepared by cloning the previously identified "ideal" PPRE sequence 5′-tgtgaccttgacctagttttg-3′ (24) into plasmid pCAT3 (Promega, Heidelberg, Germany). Transfection with 0.5 μg/well pSV-β-Gal (Promega) was performed as the internal control. After transfection, cells were incubated for 42 h with 200 μM indicated compound, dissolved in 1% Me_2SO (arachidonic acid, 100 μM). CAT and β-galactosidase concentrations were measured with an enzyme-linked immunosorbent assay detection kit (Boehringer Mannheim). Normalized CAT expression was determined and plotted as fold induction relative to untreated cells. Each experiment was performed six times with similar results.

Cloning, Expression, and Purification of GST/LBD-mPPARα Fusion Protein—The ligand binding domain of mPPARα was amplified by polymerase chain reaction from a murine liver cDNA library with a 5′ primer that introduced an *Eco*RI site and a 3′ primer that introduced a *Bam*HI site downstream of the natural stop codon of mPPARα cDNA. The resulting fragment was appropriately digested and subcloned into a *Eco*RI/*Bam*HI-digested GST fusion vector (pGEX-2T, Amersham Pharmacia Biotech). GST/LBD-mPPARα expression in *Escherichia coli* strain XL-1-Blue (Stratagene) was induced by addition of isopropyl-1-thio-b-D-galactopyranoside to the growth media (0.2 mM final concentration). After culturing for 5 h, bacterial extracts were prepared by sonication (50 W, 2 × 20 s) followed by 5 freeze/thaw cycles. The fusion protein was purified on a glutathione-Sepharose 4B column as per the manufacturer's recommendations (Amersham Pharmacia Biotech).

Dietary Intervention Studies—Mice were fed a standard chow diet (Altrumin, Hannover, Germany) containing 0.8 mg/g (w/w) of various sterols, mainly cholesterol and β-sitosterol, 0.075 mg/g (w/w) of nonesterified phytol, and 0.2 mg/g (w/w) of phytanic acid. Phytol-enriched diets were prepared from these diets by adding 5 mg/g of phytol (Aldrich). Bezafibrate was added to the standard diet at a concentration of 2.5 mg/g, treatment with 9-*cis* RA was performed by daily gavage of 10 μg of 9-*cis* RA (Sigma)/g of body weight. Animals were kept individually, and food intake and body weights were monitored daily.

Ligand Binding Assay—Ligand binding to recombinant GST/LBD-mPPARα fusion protein was performed with the fluorescent fatty acid *trans*-parinaric acid (25, 26). The concentration of *trans*-parinaric acid in absolute ethanol was determined spectrophotometrically (ϵ_{310} = 84,000 M^{-1}). Protein solution (0.1 to 0.4 μM in phosphate-buffered saline) was titrated with *trans*-parinaric acid at 25 °C using a fluorescence spectrophotometer (LS 50 B, Perkin-Elmer). For excitation and emission, wavelengths of 320 and 412 nm and a slit width of 2.5 and 20 nm were used. Ethanol concentration never exceeded 1% (v/v). All binding experiments were performed at least four times, and the dilution was subtracted from original data. The binding isotherms were fitted using a nonlinear Marquardt algorithm. For competition experiments, GST/LBD-mPPARα fusion protein (0.1 to 0.4 μM in phosphate-buffered saline) was saturated with *trans*-parinaric acid, which was then displaced from the protein using various ligands dissolved in ethanol (80 to 100 μM).

RESULTS

We demonstrated recently that the loss of the Scp2 gene function led to drastically elevated phytanic acid serum concentrations accompanied by peroxisome proliferation, hypolipidemia, impaired body weight control, neuropathy, and markedly altered hepatic gene expression (14). To characterize in more detail the impact of the gene disruption on modulation of hepatic gene expression, we exposed C75Bl/6 and Scp2 (−/−) mice to a standard laboratory chow diet (low phytol diet) and to a diet supplemented with 5 mg/g of nonesterified phytol (high phytol diet). Phytol is rapidly converted into phytanic acid in both strains of mice (14). Effects on hepatic gene expression were evaluated by Northern blot analyses with liver RNA isolated from the four groups: low phytol C57Bl/6, low phytol Scp2 (−/−), high phytol C57Bl/6, and high phytol Scp2 (−/−). We selected to study four genes that comprise functionally active PPREs: ACO (23), peroxisomal bifunctional enzyme (27), peroxisomal 3-ketoacyl-CoA thiolase (28), and liver fatty acid-binding protein (29). As shown in Fig. 1*A*, expression of all of these genes was induced considerably in the two high phytol groups. Lowest expression was consistently seen in the low phytol C57Bl/6 group, followed by the low phytol Scp2 (−/−) group (1.5-to 3-fold higher) and the high phytol C57Bl/6 group (3- to 7-fold higher). The most drastic induction was evident in the high phytol Scp2 (−/−) group in whom expression was between five- (liver fatty acid-binding protein) and more than 10-fold (peroxisomal bifunctional enzyme) higher than in the low phytol C57Bl/6 group. Thus, hepatic expression of PPARα target genes seemed to parallel phytanic acid serum concentrations (Fig. 1*B*).

To exclude hormonal or strain-specific influences on PPARα-dependent gene expression (30, 31), we next investigated whether phytanic acid could also induce the expression of target genes in a cell culture model. Therefore, we incubated the rat hepatoma cell line MH1C1 with phytanic acid and examined ACO mRNA expression by Northern blot analyses. MH1C1 cells have previously been shown to retain the ability of peroxisome proliferation in response to nafenopin and to

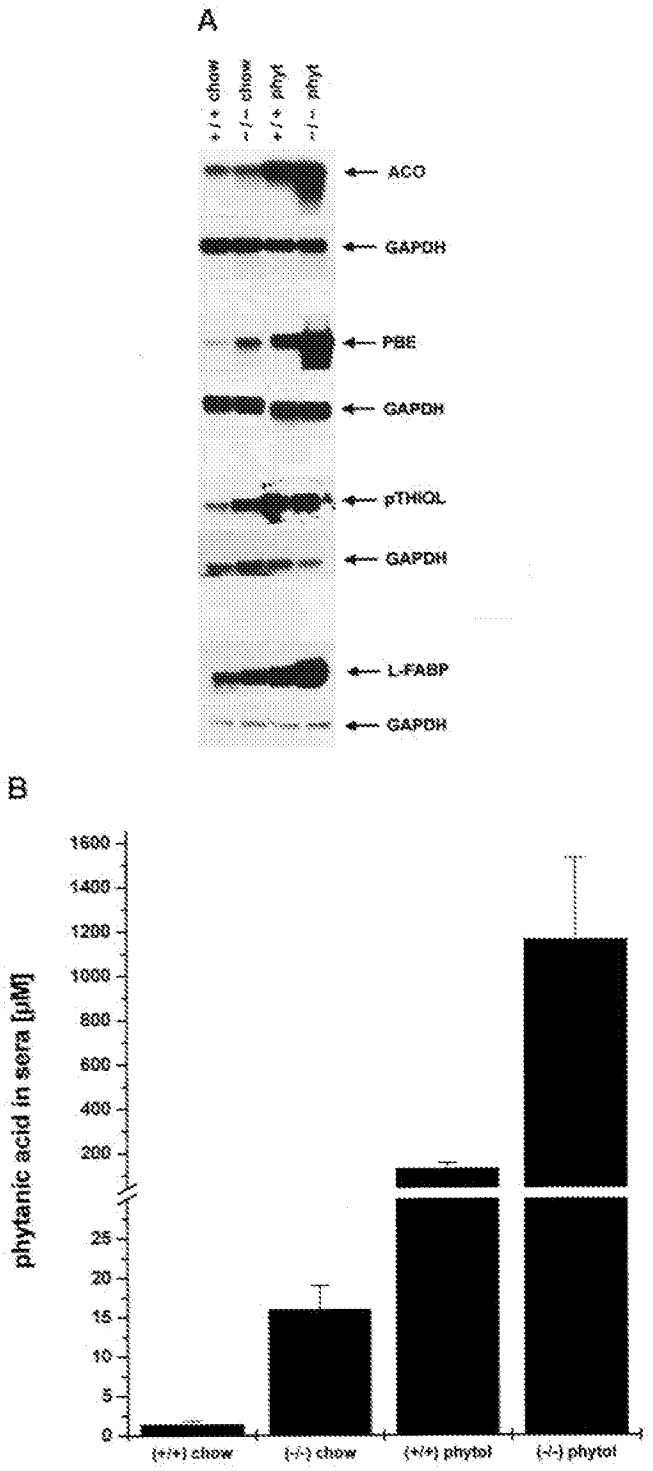

FIG. 1. **Expression of PPARα-dependent genes correlates with phytanic acid serum concentrations.** *A*, Northern blot analyses with poly(A)$^+$ RNA from liver of Scp2 (−/−) and wild type mice (+/+) fed a standard chow diet or a diet supplemented with 5 mg/g of phytol (*phyt*). The abbreviations for the cDNA probes are given in the text. Northern blots were reprobed with rat glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) cDNA to exclude lane-loading differences. *PBE*, peroxisomal bifunctional enzyme; *pTHIOL*, peroxisomal 3-ketoacyl-CoA thiolase; *L-FABP*, liver fatty acid binding protein. *B*, phytanic acid concentrations in sera from Scp (−/−) and wild type (+/+) mice. Results are expressed in µmol/liter. Quantification and identification was performed as described previously (11).

express significant amounts of PPARα (32). In accordance with our *in vivo* findings in Scp2 (−/−) mice, we found a 3- to 4-fold elevated ACO mRNA expression after incubation of MH1C1

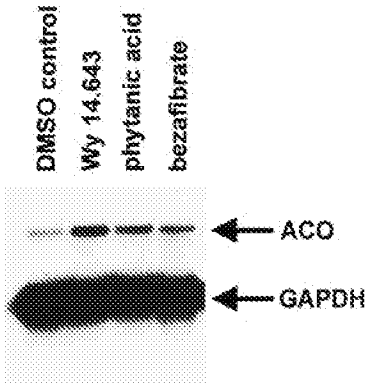

FIG. 2. **Induction of acyl-CoA oxidase mRNA in the rat hepatoma cell line MH1C1.** Cells were incubated for 72 h with 250 µM of the indicated compound dissolved in 0.5% v/v Me_2SO (*DMSO*). After isolation of poly(A)$^+$ RNA, acyl-CoA oxidase mRNA was detected by Northern blot analyses with a digoxigenin-labeled cDNA probe. Rehybridization with rat glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) cDNA was performed to exclude lane-loading differences.

cells with 250 µM phytanic acid for 3 days (Fig. 2). The increase on ACO mRNA expression was more pronounced than that obtained after incubation of this cell line with 250 µM bezafibrate (2- to 3-fold) but less prominent than that obtained with 250 µM Wy 14,643 (4- to 5-fold) (Fig. 2).

To gain further insights into the mechanism of phytol-induced modulation of gene expression, we treated Scp2 (−/−) and C57Bl/6 mice with bezafibrate and 9-*cis* RA and compared ACO gene expression in their livers with the corresponding effects of dietary phytol administration. Bezafibrate has been demonstrated to be an activator of PPARα (9), whereas 9-*cis* RA was identified as a weak activator of RXRα (17, 18). As evident from Fig. 3, treatment of Scp2 (−/−) and control mice with 9-*cis* RA alone stimulated ACO gene expression only very moderately, leading to a 1.5-fold increase that was not statistically significant. Most efficient stimulation of ACO gene expression was observed in Scp2 (−/−) mice that had been treated with either phytol or bezafibrate (Fig. 3). However the simultaneous administration of 9-*cis* RA and bezafibrate to Scp2 (−/−) and control mice did not lead to a synergistically enhanced ACO gene expression that was observed in rat hepatocyte cultures (19, 20).

These results pointed to similarities that seemed to exist between the effects of dietary phytol intake and treatment with bezafibrate. The good correlation between plasma phytanic acid concentrations and expression of PPARα target genes led to our hypothesis that this fatty acid may act as a direct agonist of PPARα, especially as it has been demonstrated that a broad range of fatty acids binds to and thereby activates this transcription factor (3, 7–10). To evaluate this hypothesis, we tested binding of phytanic acid to a recombinant glutathione-*S*-transferase/murine PPARα ligand binding domain fusion protein (GST/LBD-mPPARα) and compared its affinity with a number of well characterized PPARα activators. We used a fluorescence binding assay in which increasing concentrations of *trans*-parinaric acid were incubated with a constant amount of GST/LBD-mPPARα fusion protein. The assay takes advantage of the known fact that binding of *trans*-parinaric acid to proteins changes its spectral properties, leading to sensitized fluorescence with a maximum at a wavelength of 412 nm (excitation at 320 nm) (24). As is evident from Fig. 4*A*, saturable binding of *trans*-parinaric acid to the purified GST/LBD-mPPARα fusion protein could be demonstrated. In contrast, *trans*-parinaric acid did not bind to purified recombinant GST, thus excluding the possibility that the GST part of the fusion protein contributed significantly to the binding activity (26).

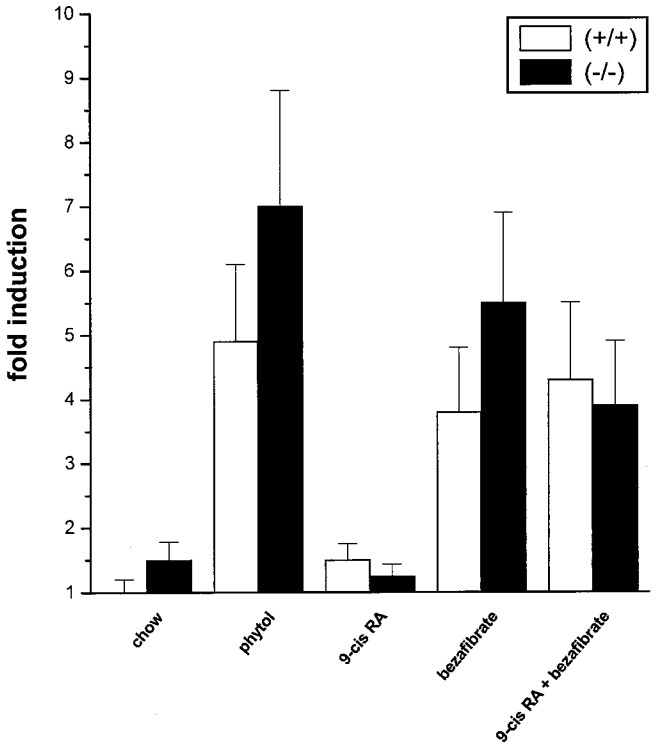

FIG. 3. **Induction of acyl-CoA oxidase mRNA in Scp2 (−/−) and wild type mice (+/+).** Mice of both strains were fed with a diet containing 5 mg/g phytol or 2.5 mg/g bezafibrate as described under "Experimental Procedures." 9-*cis* RA was administered by daily intraperitoneal gavage of 10 μg/g of body weight. Poly(A)$^+$ RNA from liver was isolated, and Northern blot analyses were performed as described in Fig. 2. Results are given in x-fold induction above basal mRNA expression level observed in wild type mice fed a standard chow diet (control).

To compare the binding affinities of several known PPARα activators with that of phytanic acid, we performed competition experiments. As shown in Fig. 4*B*, Wy 14,643 revealed the best displacement of *trans*-parinaric acid from GST/LBD-mPPARα fusion protein and thus the highest binding affinity. Surprisingly, the natural branched chain fatty acid phytanic acid bound to recombinant mPPARα far better than the well known PPARα activators bezafibrate, arachidonic acid, and palmitic acid (7, 9). In accordance with previous studies demonstrating that erucic acid does not activate PPARα (7, 10), we observed no displacement of *trans*-parinaric acid from GST/LBD-mPPARα fusion protein after adding this very long chain fatty acid and, thus, no binding.

The ability of phytanic acid to induce the expression of a CAT reporter gene linked to a PPRE was examined by co-transfection of HepG2 cells with a mPPARα-expressing plasmid (5, 23). The addition of PPARα ligands to the culture medium at a concentration of 200 μM (arachidonic acid, 100 μM) revealed a strong correlation between the binding affinity of the compounds toward mPPARα and their respective *trans*-activation ability. The administration of Wy 14,643 led to a 10-fold increase in CAT expression, followed by phytanic acid (6.5-fold), bezafibrate (4.0-fold), arachidonic acid (3.1-fold), and palmitic acid (2.2-fold) (Fig. 5). Therefore, phytanic acid is not only a high affinity ligand but also a potent activator of murine PPARα.

DISCUSSION

In a previous study, we demonstrated that Scp2 (−/−) mice had a defect in peroxisomal catabolism of phytanoyl-CoA (14). The data pointed to a dual role played by the two *Scp2*-encoded gene products, SCP2 and SCPx, which are both localized in peroxisomes as follows. 1) Reduced peroxisomal phytanoyl-CoA import seemed to relate to the absence of phytanoyl-CoA carrier function that was shown to be associated with SCP2. 2) Defective thiolytic cleavage of 3-ketopristanoyl-CoA was apparently because of absence of the 3-ketopristanoyl-CoA thiolase activity that was shown to be associated with SCPx (13, 33, 34). In addition to the metabolic defect, we observed profound peroxisome proliferation, hypolipidemia, and increased expression of genes encoding proteins that function in peroxisomal and mitochondrial β-oxidation (14). The purpose of the present work was to characterize the latter effects of the gene disruption in more detail.

In vitro data published earlier (17, 18) showed that phytanic acid behaves like a weak activating ligand of RXRα and thus may act as 9-*cis* RA-like agonist when present in high concentrations. Because RXRα is an obligatory partner in PPRE-dependent gene expression (2, 3), we initially considered that the effects on gene expression in Scp2 (−/−) mice were because of enhanced activation of RXRα in this transgenic model. However, the evidence that we present in the current manuscript does not support this hypothesis, as follows. 1) Application of 9-*cis* RA to control mice did not induce ACO gene expression, although 9-*cis* RA has been demonstrated to be a more potent activator of RXRα than phytanic acid. 2) Application of the RXRα agonist 9-*cis* RA to both strains of mice did not evoke hypotriglyceridemia or peroxisome proliferation, [2] which were observed in Scp2 (−/−) mice, especially after feeding the phytanic acid precursor phytol. Therefore, it seems unlikely that the effects observed in Scp2 (−/−) mice are because of phytanic acid-induced activation of RXRα.

Because a broad range of fatty acids has been shown to activate PPARα *in vitro* (3, 7–10), we investigated whether the enhanced hepatic gene expression in our mouse model could be because of the phytanic acid-induced activation of PPARα. It has been demonstrated that ligand binding to PPARα induces a conformational change that enables the protein to interfere with basal transcription machinery (35). The DNA binding affinity of PPARα is also enhanced in the presence of ligands, at least if the receptor concentration is limiting (7). Therefore, ligand binding is a necessary prerequisite for the activation of PPARα-dependent gene expression. We measured the ability of phytanic acid and several well known PPARα activators to bind to a recombinant GST/LBD-mPPARα fusion protein. So far, ligands of murine and *Xenopus* PPARα have been primarily identified by indirect binding assays, in which the ligand-dependent DNA binding activity of PPARα (7) or the ligand-induced activation of coactivator proteins (9) were measured. K_d-values have only been reported for the few cases in which radiolabeled ligands were available (10, 36). The *trans*-parinaric acid competition assay that we used in the present study allowed us to identify direct binding of ligands to the soluble GST/LBD-mPPARα fusion protein. Furthermore, actual K_d-values for the ligands could be obtained using a Marquardt algorithm. For Wy 14,643, a K_d value of 4 nM was calculated, followed by phytanic acid (10 nM), bezafibrate (45 nM), arachidonic acid (83 nM), and palmitic acid (100 nM).

The affinities for straight chain fatty acids in binding to PPARα were found in the range of their respective physiological serum concentrations (~30 μM) (7, 10). Because phytanic acid bound to the recombinant GST/LBD-mPPARα fusion protein with at least one order of magnitude higher affinity than palmitic acid, one might consider that this dietary fatty acid also binds within its physiological serum concentration range (1.3–6.5 μM) (17). The direct binding of phytanic acid to recom-

[2] U. Seedorf, unpublished observation.

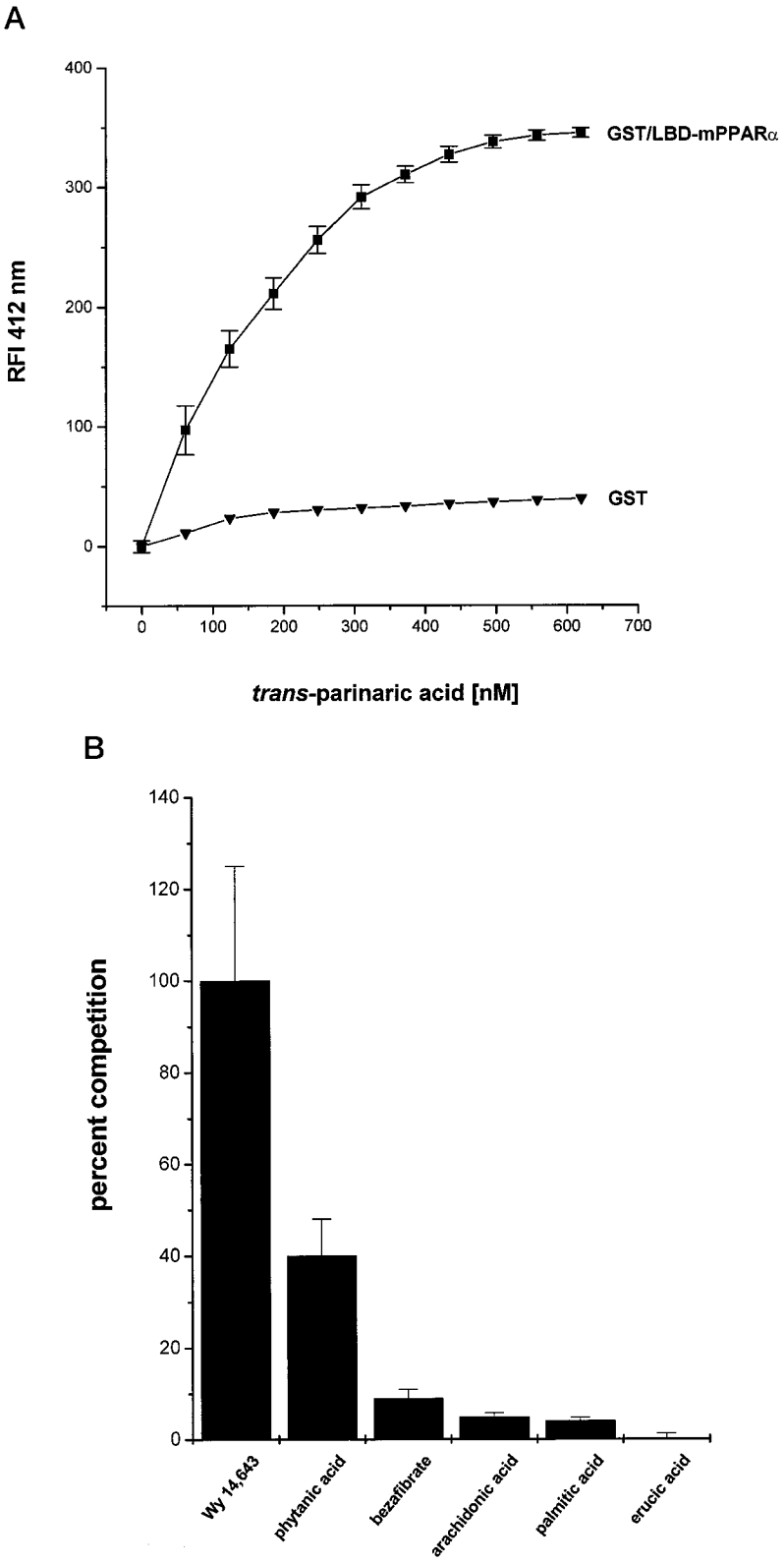

FIG. 4. *A*, **binding of *trans*-parinaric acid to a recombinant expressed GST/LBD-mPPARα fusion protein.** GST/LBD-mPPARα fusion protein or GST alone were dissolved in phosphate-buffered saline (0.1 to 0.4 μM) and titrated with *trans*-parinaric acid. Emission was monitored using a fluorescence spectrophotometer at a wavelenght of 412 nm (excitation wavelenght 320 nm). *B*, displacement of *trans*-parinaric acid bound to GST/LBD-mPPARα fusion protein by peroxisome proliferators and fatty acids. The fusion protein was saturated with *trans*-parinaric acid followed by the addition of various ligands dissolved in ethanol. Results are given in percent displacement compared with Wy 14,643 (= 100%).

binant PPARα supports the assumption that PPARα activation is not necessarily achieved by a common endogenous ligand that mediates the effects of the structural diverse PPARα activators. The binding affinities of the compounds toward the recombinant GST/LBD-mPPARα fusion protein corresponded well with their *trans*-activation ability, obtained by co-transfection of a PPRE-driven CAT reporter gene and a mPPARα-expressing plasmid into HepG2 cells. In addition, the extent of induction of acyl-CoA oxidase mRNA expression in MH1C1 cells was also consistent with the *trans*-activation ability of the compounds.

For several reasons, the identification of phytanic acid as a PPARα agonist is of special interest. First, phytanic acid does not only accumulate in Scp2 (−/−) mice but also in several inherited human diseases like Refsum disease and Zellweger syndrome (21). Although remarkable differences were observed in the ligand binding affinities between rodent and human PPARα (37), we found that phytanic acid binds to recombinant

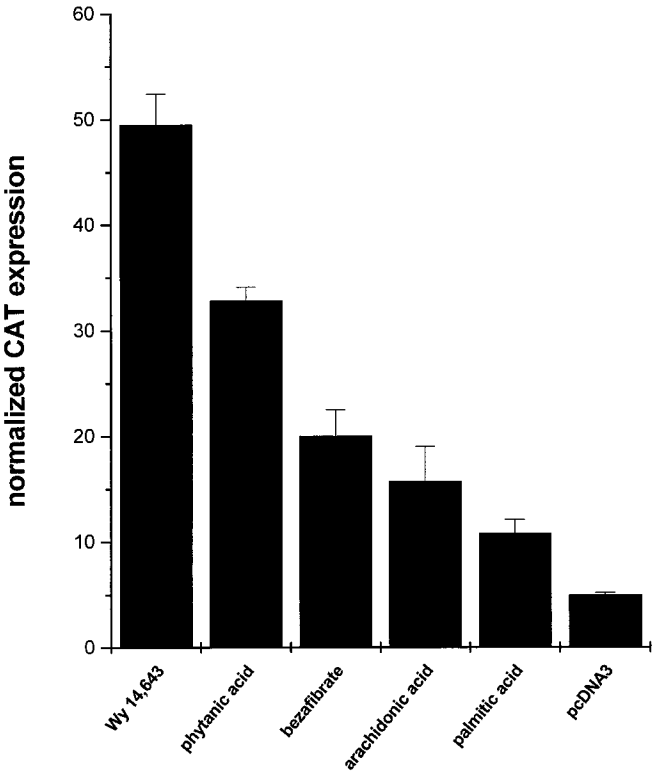

FIG. 5. **Activation of a CAT reporter gene linked to a PPRE.** The *pCAT-iPPRE* reporter gene construct was co-transfected with a mPPARα-expressing vector in the presence of a β-galactosidase control vector. Cells were incubated for 42 h with 200 μM indicated compound, dissolved in 1% Me_2SO (arachidonic acid, 100 μM). CAT and β-galactosidase expression was measured from cell lysates of treated and untreated cells. Results are given in normalized CAT expression relative to untreated cells.

human PPARα with a comparable affinity as to murine PPARα.[3] Second, phytanic acid is the first identified natural PPARα ligand that is primarily degraded in peroxisomes (21, 38). So far, a variety of endogenous fatty acids have been described as PPARα activators without being substrates for peroxisomal degradation. On the other hand, very long chain fatty acids that are primarily degraded by peroxisomal β-oxidation neither bind to nor activate PPARα (7). Therefore, one might consider that phytanic acid induces its own degradation via activation of the PPARα-dependent peroxisomal oxidation pathways. However, because of its β-methyl group, phytanic acid cannot be degraded by β-oxidation. Instead, a one carbon moiety is split from the molecule by α-oxidation, yielding pristanic, which is then subjected to six cycles of peroxisomal β-oxidation (21, 39). We demonstrated previously that the expression of the key step enzyme in phytanic acid α-oxidation, phytanoyl-CoA hydroxylase, is not increased in Scp2 (−/−) mice, although the serum phytanic acid concentrations increases up to 1000-fold after phytol feeding (14). This leads to the conclusion that the initial step in phytanic acid degradation is not regulated by the substrate concentration. Instead, we consider that phytanic acid serves as a dietary signal molecule that induces the catabolism of fatty acids by activating PPARα. This assumption is supported by the recent finding that PPARα also modulates constitutive expression of genes encoding several mitochondrial fatty acid-catabolizing enzymes (40). The dietary uptake of physiological concentrations of phytanic acid together with a bulk of other fatty acids would lead to an enhanced mitochondrial and peroxisomal β-oxidation because of the activation of PPARα. This is in accordance with our previous findings, which in addition to the peroxisomal β-oxidation enzymes, the expression of mitochondrial 3-ketoacyl-CoA thiolase mRNA as well as enzymatic activity of mitochondrial butyryl-CoA dehydrogenase is drastically enhanced in the liver of Scp2 (−/−) mice (14). The phytanic acid-induced expression of genes encoding mitochondrial and peroxisomal β-oxidation enzymes might also explain the observed hypolipidemia in Scp2 (−/−) mice (14). Therefore, phytanic acid could serve as a dietary signal leading to the induction of fatty acid catabolism.

[3] C. Wolfrum, unpublished observation.

Acknowledgment—We thank P. R. Holden (Zeneca, Macclesfield, Cheshire) for providing plasmid pcDNA3-mPPARα and B. Glass and S. Lütke-Enking for expert technical assistance.

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